

University of Groningen

Multiple effects of differentiation-inducing factor on prespore differentiation and cyclic-AMP signal transduction in *Dictyostelium*

Wang, Mei; Haastert, Peter J.M. van; Schaap, Pauline

Published in:
Differentiation

DOI:
[10.1111/j.1432-0436.1986.tb00406.x](https://doi.org/10.1111/j.1432-0436.1986.tb00406.x)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1986

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Wang, M., Haastert, P. J. M. V., & Schaap, P. (1986). Multiple effects of differentiation-inducing factor on prespore differentiation and cyclic-AMP signal transduction in *Dictyostelium*. *Differentiation*, 33(1), 24-28. <https://doi.org/10.1111/j.1432-0436.1986.tb00406.x>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Multiple effects of differentiation-inducing factor on prespore differentiation and cyclic-AMP signal transduction in *Dictyostelium*

Mei Wang*, Peter J.M. Van Haastert, and Pauline Schaap

Cell Biology and Morphogenesis Unit, Zoological Laboratory, University of Leiden, Kaiserstraat 63, NL-2311 GP Leiden, The Netherlands

Abstract. The effects of the differentiation inducing factor (DIF) on several cAMP-induced responses in *Dictyostelium* were investigated. It was found that DIF reduces the apparent affinity of cell-surface cAMP receptors. DIF does not affect the cAMP-induced cGMP response, but it is a potent inhibitor of the cAMP-relay response. DIF also inhibits the induction of prespore differentiation by cAMP in aggregation-competent cells. We also compared the effects of DIF on cAMP-induced responses with those of the relay inhibitor, caffeine, and the morphogen, adenosine.

Introduction

During the development of *Dictyostelium discoideum*, cAMP functions both as a morphogen and as a chemoattractant. Upon depletion of food, some cells start to secrete pulses of cAMP. Surrounding cells detect the cAMP signal by means of cell surface receptors and respond with a short transient increase of intracellular cGMP levels [10, 39] and transient synthesis and secretion of cAMP [16, 23]. The cGMP response is most likely involved in transduction of the cAMP signal to chemotaxis [10, 17, 34, 39], while the cAMP response causes relay of the initial cAMP pulse to more distally located cells [see 4]. The cells aggregate to form multicellular structures, which assume the shape of a slug and ultimately culminate to form a fruiting body consisting of stalk cells and spores. Cell movement in the multicellular stage is controlled by a small group of cells, the tip [15], which continue to secrete cAMP pulses autonomously [19, 21].

Spore specific gene expression occurs shortly after completion of aggregation [1, 12, 13]. This type of gene expression can be induced by micromolar cAMP concentrations [11, 18, 27]; this effect of cAMP is also mediated by surface cAMP receptors [18]. Micromolar cAMP concentrations are considered to accumulate in multicellular structures by continued cAMP signalling [18, 20].

Three other compounds produced by *D. discoideum* are known to control cell type specific differentiation. Firstly, a differentiation inducing factor, DIF, induces stalk cell differentiation in submerged monolayers of *D. discoideum* V12M2 [3, 8] and in *D. discoideum* NC-4 slugs (unpublished results). DIF also inhibits prespore differentiation in submerged monolayers and in slugs [8]. Secondly, ammonia,

an end-product of protein degradation in *D. discoideum*, promotes spore differentiation in submerged monolayers of V12M2 [7]. Ammonia was also reported to inhibit the cAMP relay response [39]. Thirdly, adenosine, a cAMP hydrolysis product prevents the conversion of prestalk into prespore cells [37] and inhibits cAMP induced prespore differentiation [20]. Adenosine also inhibits cAMP relay, the cAMP induced cGMP response and the binding of cAMP to surface cAMP receptors [14, 25, 28]. The inhibition of cAMP binding is considered to be the cause of the inhibition by adenosine of cAMP induced prespore differentiation and cAMP relay [25, 35].

In this study we investigated the effects of DIF on cAMP binding and several cAMP induced responses. We found that DIF inhibits cAMP induced prespore differentiation and reduces the apparent affinity of the surface cAMP receptor. DIF does not affect the cGMP response, but is a potent inhibitor of the cAMP relay response.

Methods

Materials

cAMP, 2'-deoxy-adenosine 3'-5'-monophosphate (dcAMP), and dithiothreitol (DTT) were obtained from Sigma (St. Louis, USA); 2,8-³H-cAMP and cGMP radioimmunoassay (RIA) kits were purchased from Amersham (UK). Lichrosorb 10RPB was obtained from Chrompack Middelburg (The Netherlands). The MUD-1 monoclonal antibody [5] was kindly supplied by Dr. Marianne Krefft (Wuppertal, FRG).

Culture conditions

Dictyostelium discoideum NC-4 was cultured in association with *Escherichia coli* 281 on glucose/peptone agar [28]. Vegetative cells were separated from the bacteria, placed on nonnutrient agar at a density of 2.5×10^6 cells/cm², and incubated for 16–20 h at 6° C. After this, the cells had segregated into aggregation territories; they were fully aggregation competent, but had not yet started to aggregate. The cells were harvested and resuspended in 10 mM Na/K phosphate buffer (PB) pH 6.5.

Purification and assay of DIF

DIF was purified from *D. discoideum* AX-2 cells essentially according to the procedure described by Kay et al. [9], ex-

* To whom offprint requests should be sent

cept that a Lichrosorb-10RPB column was used for both high-performance liquid chromatography (HPLC) purification steps. In the second HPLC step, isocratic elution using 50% acetonitrile and 5% acetic acid was applied instead of gradient elution. The eluent of the second HPLC purification step contained several fractions exhibiting DIF activity. We used the fraction exhibiting the highest peak of DIF activity, which according to Kay et al. [9], is DIF-1. Three independently isolated and purified DIF preparations were used and gave similar results.

Effects of DIF on cAMP binding, cAMP relay, and cGMP response

Aggregation-competent cells that had been resuspended in PB at a density of 5×10^7 cells/ml were aerated for 10 min and then incubated with various amounts of DIF for 10–16 min at 22° C. cAMP relay was measured by stimulating 13.5- μ l aliquots of DIF-treated cells with 1.5 μ l 50 μ M dcAMP and 50 mM DTT in PB. After 2 min, the cells were lysed with perchloric acid, and the cAMP content of the neutralized lysate was determined using a cAMP-isotope dilution assay [6, 30].

The cGMP response was measured by stimulating similar amounts of DIF-treated cells with 0.1 μ M cAMP (final concentration) and lysing the cells 10 s after the addition of the stimulus [33]. The level of cGMP was measured using a commercially available cGMP RIA kit.

The effects of DIF on cAMP binding were measured by two different methods. In the first method 40- μ l aliquots of DIF-treated cells were incubated for 45 s at 20° C with 10 μ l 3 H-cAMP at various concentrations and 5 mM DTT. The cells were then separated from the incubation mixture by centrifugation through silicone oil, and the radioactivity of the pellet was measured [32]. In the second method, 40- μ l aliquots were incubated for 1 min at 0° C with various concentrations of 3 H-cAMP and 5 mM DTT. Subsequently, 0.5 ml saturated ammonium sulfate and 25 μ l 10 mg/ml bovine serum albumin were added. After a further 5 min of incubation at 0° C, the cells were centrifuged, and the radioactivity of the pellet was measured [31].

Induction and assay of prespore differentiation

Aggregation-competent cells were suspended at a density of 10^7 cells/ml in PB, and 100- μ l aliquots were incubated at 22° C [35]. Every 60 min, 1 μ l 10^{-2} M cAMP was added to the cell suspensions. After 6 h, the cells were lysed by freezing/thawing, and the levels of the MUD-1 antigen were measured using an ELISA assay [18].

Results

Effects of DIF on cAMP-induced prespore differentiation

cAMP induces prespore differentiation in aggregation-competent cells after 6–8 h of incubation [18]. We measured the effects of different amounts of DIF on the induction of prespore-specific antigen by 0.1 and 1 mM cAMP (Fig. 1). The induction of prespore antigen by 0.1 mM cAMP was completely inhibited by DIF at a concentration of 10,000 U/ml and was reduced by about one-half by 3,000 IU/ml DIF. In the case of induction by 1 mM cAMP, the inhibitory effect of DIF was much less, which suggests

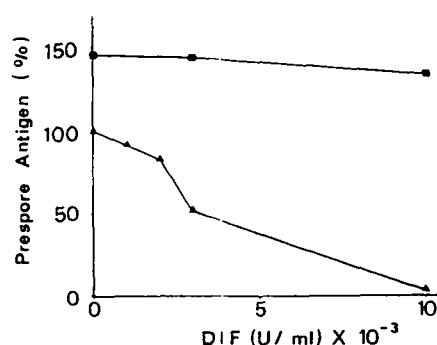


Fig. 1. Effects of DIF on cAMP-induced prespore differentiation. Aggregation-competent cells (10^7 cells/ml) were incubated for 6 h with 0.1 mM cAMP (∇) or 1 mM cAMP (\blacksquare) and 0–10,000 U DIF per milliliter. Prespore-antigen levels were measured using the MUD-1 monoclonal antibody [5]. The data are expressed as percentages of the prespore-antigen levels induced by 0.1 mM cAMP in the absence of DIF. All data are the means of the results of three experiments performed in triplicate.

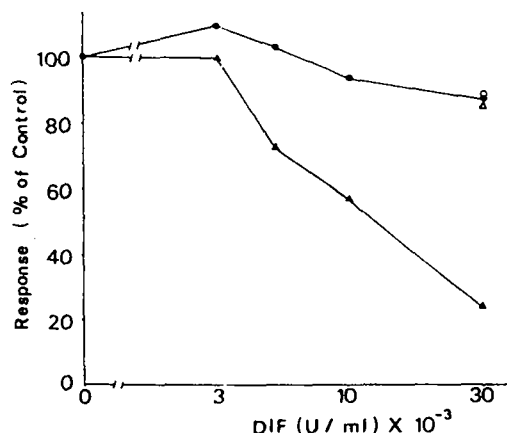


Fig. 2. Effects of DIF on cAMP relay and the cGMP response. Aggregation-competent cells (5×10^7 cells/ml) were treated for 10–15 min with 0–30,000 U DIF per milliliter. cAMP accumulation was measured 2 min after stimulation with 5 μ M dcAMP and 5 mM DTT (∇), and cGMP accumulation was measured 10 s after stimulation with 1 μ M cAMP (\bullet). The data are expressed as percentages of the cAMP or cGMP accumulation induced in the absence of DIF. cAMP (∇) and cGMP (\bullet) accumulation after treatment with the vehicle of DIF (i.e., 0.4% ethanol for 30,000 U/DIF per milliliter DIF) are also shown. The means of the results of three experiments are presented.

that the effects of cAMP and DIF on prespore differentiation are mutually antagonistic.

Effects of DIF on cAMP relay and cGMP response

Aggregation-competent cells were preincubated with DIF at concentrations of 3,000–30,000 units/ml. The cells were then stimulated with 1 μ M cAMP or 5 μ M dcAMP/DTT to allow measurement of the cGMP response and the cAMP-relay response, respectively. cGMP levels were measured 10 s after stimulation with cAMP, and cAMP levels were measured 2 min after stimulation with dcAMP/DTT (Fig. 2).

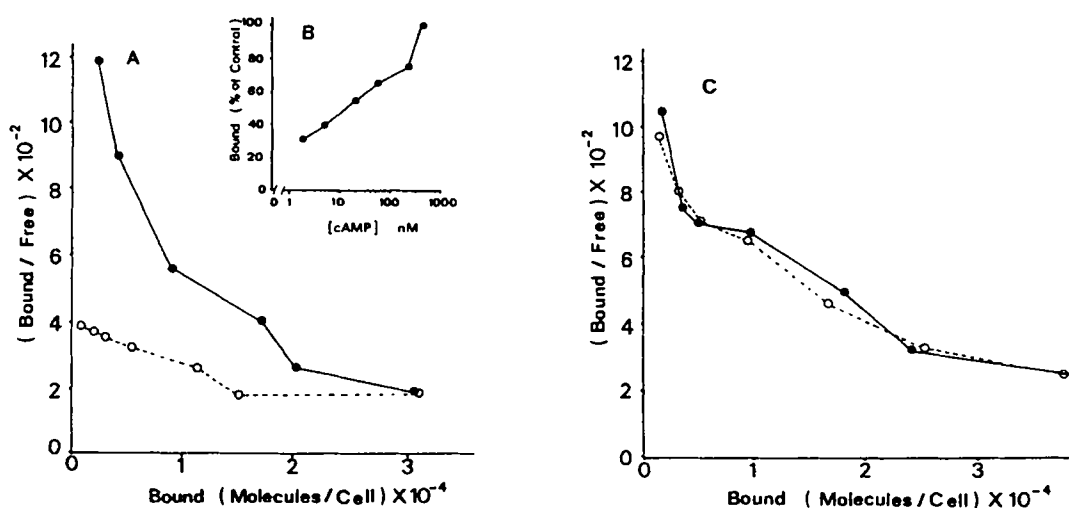


Fig. 3A–C. Effects of DIF on ^3H -cAMP binding activity. Aggregation-competent cells were incubated for 15 min in PB in the presence (○) or absence (●) of 10,000 U/DIF per milliliter. The cells were then incubated with 2–200 nM ^3H -cAMP for 1 min, and the radioactivity associated with the cell surface was measured by centrifuging the cells through silicone oil (A, B) or by precipitating the cells in ammonium sulfate (C). Scatchard plots; B the binding activity of DIF-treated cells was plotted as a percentage of the binding activity of control cells. The means of the results of two experiments are shown

DIF did not significantly affect the cGMP response. The small amount of inhibition observed at the highest DIF concentration was also induced by the vehicle of DIF, i.e., ethanol (final concentration 0.4%). In this experiment, nearly saturating concentrations of the stimulus were used. Also, at subsaturating cAMP concentrations, DIF did not affect the cGMP response (data not shown).

DIF was found to have very pronounced effects on the cAMP-relay response. DIF strongly inhibited the accumulation of cAMP induced by 5 μM dcAMP. The inhibition was about 80% at a DIF concentration of 30,000 U/ml and was 50% when 10,000 U/ml DIF was applied. Ethanol (0.4%) induced minor inhibition of cAMP relay. Preincubation with DIF did not affect the basal cAMP levels of unstimulated cells (data not shown).

Effects of DIF on cAMP binding

The effects of DIF on cAMP relay and on the cGMP response were similar to those of caffeine, which also inhibits cAMP relay, while not affecting the cGMP response [2, 24, 32]. The binding properties of cell-surface cAMP receptors appear different when measured by precipitating cells in ammonium sulfate than when cells were sedimented through silicone oil [31]. Caffeine (10 mM) induces a marked decrease in the apparent affinity of ^3H -cAMP binding when measured using ammonium sulfate, but only a small effect is observed when binding is measured using silicone oil [2, 31, 32]. In order to further compare the effects of DIF and caffeine, we measured the effects of DIF (10,000 U/ml) on the binding of 2–200 nM ^3H -cAMP using both binding assays (Fig. 3).

Surprisingly, the effects of DIF on cAMP binding were more-or-less opposite to those of caffeine. DIF was found to have no effect when binding was measured with ammonium sulfate (Fig. 3C). However, when measured with silicone oil, DIF induced marked inhibition of the binding of low concentrations of cAMP, whereas the binding of high cAMP concentrations was much less affected (Fig. 3A,

B). Scatchard plot analysis of the binding data indicated that DIF decreases the apparent affinity of ^3H -cAMP binding (Fig. 3A).

Discussion

In the present study, the effects of DIF on several cAMP-induced responses were investigated. It was found that DIF inhibits cAMP-induced prespore differentiation in aggregation-competent cells, and that it reduces the apparent affinity of cAMP for the surface receptor when measured using silicone oil, although not when measured using ammonium sulfate. DIF does not affect cAMP-induced guanylate-cyclase activation, but it is a potent inhibitor of cAMP-induced adenylate-cyclase activation: half-maximal inhibition of the latter response occurred at a DIF concentration of 10,000 U/ml, which is equivalent to about 200 nM DIF [9]. In order to exert effects on cAMP relay, cAMP binding, and cAMP-induced prespore differentiation, higher amounts of DIF are required than those necessary to affect stalk-cell differentiation, which is 50% inhibited by a DIF concentration of 50 U per 2 ml of 0.75×10^4 cells/ml [3]. This observation may be related to the fact that DIF, which is a very lipophilic compound, is taken up by cells, thus causing DIF depletion in the extracellular medium. Calculated on a per cell basis, half-maximal inhibition of stalk-cell differentiation occurs at a DIF concentration of 6,500 U per 10^6 cells, and the same degree of inhibition of cAMP relay and cAMP-induced prespore differentiation is produced by concentrations of 200 and 300 IU per 10^6 cells, respectively.

Causal relationships between DIF-inhibited responses

The activation of adenylate cyclase by cAMP involves the binding of cAMP to surface receptors [26] and, probably, the interaction of receptors with a guanine-nucleotide regulatory protein [29] as well as the interaction of this protein with adenylate cyclase (unpublished results). It is unlikely that DIF inhibits cAMP relay solely by reducing the appar-

Table 1. Effects of adenosine, caffeine, and DIF on cAMP-induced responses and morphogenesis

Response	Adenosine	Caffeine	DIF
cAMP binding:			
silicone oil	— [14]	0/— [2, 32]	—
ammonium sulfate	— [25, 35]	— [31]	0
cAMP relay:			
excitation	— [24, 25]	— [2, 24]	—
adaptation	— [24, 25]	0 [24]	ND
cGMP response	— [28]	0/+ [2, 32]	0
cAMP-induced prespore differentiation	— [20]	0/— [22]	—
Stalk-cell differentiation	0*	ND	+ [3, 8]
Multiple tips (cAMP oscillators)	— [20]	+* [36]	+*

+, induces or promotes; —, inhibits; 0, no effect; ND, not determined. Square brackets indicate references

* Unpublished data

ent affinity of the cAMP receptor, because the relay response was measured using a saturating concentration of the stimulus. It has previously been shown that the affinity of cAMP receptors is modulated by guanine nucleotides [29]. It is possible that DIF alters the receptor G-protein or G-protein/adenylate-cyclase interaction, resulting in both the inhibition of adenylyl-cyclase activation and the reduction of receptor affinity.

The effects of DIF on cAMP binding are probably not responsible for the inhibition of cAMP-induced prespore differentiation, since DIF specifically inhibits the binding of nanomolar cAMP concentrations (Figs. 3A, B), while prespore differentiation is induced by supramicromolar cAMP concentrations (Fig. 1). It is also unlikely that the inhibition of cAMP relay by DIF is directly responsible for the inhibition of cAMP-induced prespore differentiation, since high concentrations of another relay inhibitor, caffeine [2, 24], induce only minor inhibition of prespore differentiation [22]. Furthermore, prespore differentiation is induced by cAMP in a mutant in which the cAMP-relay response is virtually absent [22].

In the physiological situation, i.e., prespore differentiation in slugs, the inhibitory effect of DIF on cAMP relay may indirectly inhibit prespore differentiation by decreasing cAMP levels in the slug.

Comparison of the effects of DIF, caffeine, and adenosine

The effects of DIF resemble, in some respects, the effects of the relay inhibitor, caffeine [2, 24], as well as those of the morphogen, adenosine [20, 37]. The effects of DIF, caffeine, and adenosine on cAMP-induced responses, stalk-cell differentiation, and tip formation are summarized in Table 1.

As is the case for DIF, caffeine inhibits cAMP relay [2, 24], it does not affect or slightly potentiates the cGMP response [2, 32], and it induces multiple tip formation ([36]; unpublished results). In contrast to DIF, high concentrations of caffeine (10 mM) strongly reduce the affinity of cAMP binding when measured using ammonium sulfate,

but only weakly when measured using silicone oil [2, 31]. Caffeine exerts only a minor inhibitory effect on cAMP-induced prespore differentiation [22]. It has been suggested that the effect of caffeine on cAMP relay, which occurs at a concentration of 1 mM, is not due to inhibition of cAMP binding but is the result of the inhibition of a step occurring between the cAMP receptor and adenylyl-cyclase activation [24].

Adenosine inhibits cAMP-induced prespore differentiation and cAMP relay [20, 25]. Adenosine inhibits cAMP binding, but in contrast to DIF, the inhibition of binding is evident in both PB and ammonium sulfate [14, 25, 28]. Additional differences between adenosine and DIF are that adenosine also inhibits the cGMP response [28], it does not induce stalk-cell differentiation in submerged monolayers of *D. discoideum* V12M2 (unpublished data), and it reduces the numbers of tips (autonomous cAMP oscillators) on aggregates [20], whereas DIF induces multiple tip formation (unpublished results). In contrast to caffeine, which inhibits only excitation, adenosine inhibits both the excitation and adaptation of the cAMP-relay response [24, 25]. Since adenosine inhibits cAMP binding under all tested conditions and inhibits all investigated responses to cAMP that are mediated by surface cAMP receptors, the effects of adenosine on cAMP relay, the cGMP response, and cAMP-induced prespore differentiation are considered to be a direct consequence of the inhibition of cAMP binding by adenosine [14, 25, 28, 35].

Two important morphogens in *Dictyostelium* – DIF and adenosine – interfere with the transduction of cAMP in a specific manner. Adenosine probably acts by inhibiting cAMP binding, while DIF may interact with the functional coupling between the cAMP receptor and target proteins involved in further signal transduction. Since the specific effects of these compounds on morphogenesis and cell-type-specific differentiation may largely result from interactions with cAMP-signal transduction, further analysis of the transduction of cAMP to gene expression as well as of the modulating effects of DIF and adenosine on cAMP transduction pathway(s) is required.

Acknowledgements. We are grateful to Dr. Marianne Krefft for her kind gift of the MUD-1 monoclonal antibody, and to Dr. Robert R. Kay for an initial gift of DIF. We further thank Theo M. Konijn for critically reading the manuscript. This research was supported by the C. and C. Huygens Fund, which is subsidized by the Netherlands Organisation for the Advancement of Pure Scientific Research.

References

1. Barklis E, Lodish HF (1983) Regulation of *Dictyostelium discoideum* mRNAs specific for prespore and prestalk cells. *Cell* 32:1139–1148
2. Brenner M, Thoms SD (1984) Caffeine blocks activation of cyclic AMP synthesis in the cellular slime mold *Dictyostelium discoideum*. *Dev Biol* 101:136–146
3. Brookman JJ, Town CD, Jermyn KA, Kay RR (1982) Developmental regulation of a stalk cell differentiation-inducing factor in *Dictyostelium discoideum*. *Dev Biol* 91:191–196
4. Devreotes PN (1983) Cyclic nucleotides and cell-cell communication in *Dictyostelium discoideum*. *Adv Cyclic Nucleotide Res* 15:55–96
5. Gregg JH, Krefft M, Haas-Kraus A, Williams KL (1982) Antigenic differences detected between prespore cells of *Dictyoste-*

- lium discoideum* and *Dictyostelium mucoroides* using monoclonal antibodies. *Exp Cell Res* 142:229–233
6. Gilman AG (1970) A protein binding assay for adenosine 3', 5'-cyclic monophosphate. *Proc Natl Acad Sci USA* 67:305–312
 7. Gross JD, Bradbury J, Kay RR, Peacey MJ (1983) Intracellular pH and the control of cell differentiation in *Dictyostelium discoideum*. *Nature* 303:244–245
 8. Kay RR, Jermyn KA (1983) A possible morphogen controlling differentiation in *Dictyostelium*. *Nature* 303:242–244
 9. Kay RR, Dhokia B, Jermyn KA (1983) Purification of stalk-cell-inducing morphogens from *Dictyostelium discoideum*. *Eur J Biochem* 136:51–56
 10. Mato JM, Krens FA, Van Haastert PJM, Konijn TM (1977) 3':5'-Cyclic AMP-dependent 3':5'-cyclic GMP accumulation in *Dictyostelium discoideum*. *Proc Natl Acad Sci USA* 74:2348–2351
 11. Mehdy MC, Firtel RA (1985) A secreted factor and cyclic AMP jointly regulate cell-type-specific gene expression in *Dictyostelium discoideum*. *Mol Cell Biol* 5:705–713
 12. Mehdy MC, Ratner D, Firtel RA (1983) Induction and modulation of cell-type-specific gene expression in *Dictyostelium*. *Cell* 32:763–771
 13. Morrissey JH, Devine KM, Loomis WF (1984) Timing of cell-type-specific differentiation in *Dictyostelium discoideum*. *Dev Biol* 103:414–424
 14. Newell PC, Ross FM (1982) Inhibition by adenosine of aggregation centre initiation and cyclic AMP binding in *Dictyostelium*. *J Gen Microbiol* 128:2715–2724
 15. Raper KB (1940) Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J Elisha Mitchell Sci Soc* 56:241–282
 16. Roos W, Nanjundiah V, Malchow D, Gerisch G (1975) Amplification of cyclic-AMP signals in aggregating cells of *Dictyostelium discoideum*. *FEBS Lett* 53:139–142
 17. Ross FM, Newell PC (1981) Streamers: Chemotactic mutants of *Dictyostelium discoideum* with altered cGMP metabolism. *J Gen Microbiol* 127:339–350
 18. Schaap P, Van Driel R (1985) The induction of post-aggregative differentiation in *Dictyostelium discoideum* by cAMP. Evidence for the involvement of the cell surface cAMP receptor. *Exp Cell Res* 159:388–398
 19. Schaap P, Wang M (1984) The possible involvement of oscillatory cAMP signaling in multicellular morphogenesis of the cellular slime molds. *Dev Biol* 105:470–478
 20. Schaap P, Wang M (1986) Interactions between adenosine and oscillatory cAMP signaling regulate size and pattern in *Dictyostelium*. *Cell* 45:137–144
 21. Schaap P, Konijn TM, Van Haastert PJM (1984) cAMP pulses coordinate morphogenetic movement during fruiting body formation of *Dictyostelium minutum*. *Proc Natl Acad Sci USA* 81:2122–2126
 22. Schaap P, Van Lookeren Campagne MM, Van Driel R, Spek W, Van Haastert PJM, Pinas J (1986) Postaggregative differentiation induction by cyclic AMP in *Dictyostelium*. Intracellular transduction pathway and requirement for additional stimuli. *Dev Biol* (in press)
 23. Shaffer BM (1975) Secretion of cyclic AMP induced by cyclic AMP in the cellular slime mould *Dictyostelium discoideum*. *Nature* 255:549–552
 24. Theibert A, Devreotes PN (1983) Cyclic 3',5'-AMP relay in *Dictyostelium discoideum*: Adaptation is independent of activation of adenylate cyclase. *J Cell Biol* 97:173–177
 25. Theibert A, Devreotes PN (1984) Adenosine and its derivatives inhibit the cAMP signaling response in *Dictyostelium discoideum*. *Dev Biol* 106:166–173
 26. Theibert A, Palmisano M, Jastorff B, Devreotes PN (1986) The specificity of the cAMP receptor mediating activation of adenylate cyclase in *Dictyostelium discoideum*. *Dev Biol* 144:529–533
 27. Town C, Gross J (1978) The role of cyclic nucleotides and cell agglomeration in postaggregative enzyme synthesis in *Dictyostelium discoideum*. *Dev Biol* 63:412–420
 28. Van Haastert PJM (1983) Binding of cAMP and adenosine derivatives to *Dictyostelium discoideum* cells. Relationships of binding, chemotactic and antagonistic activities. *J Biol Chem* 258:9643–9648
 29. Van Haastert PJM (1984) Guanine nucleotides modulate cell surface cAMP-binding sites in membranes from *Dictyostelium discoideum*. *Biochem Biophys Res Commun* 124:597–604
 30. Van Haastert PJM (1984) A method for studying cAMP-relay in *Dictyostelium discoideum*: The effect of temperature on cAMP-relay. *J Gen Microbiol* 130:2559–2564
 31. Van Haastert PJM (1985) The modulation of cell surface cAMP receptor from *Dictyostelium discoideum* by ammonium sulfate. *Biochim Biophys Acta* 845:254–260
 32. Van Haastert PJM, De Wit RJW (1984) Demonstration of receptor heterogeneity and affinity modulation by nonequilibrium binding experiments. The cell surface cAMP receptor of *Dictyostelium discoideum*. *J Biol Chem* 259:13321–13328
 33. Van Haastert PJM, Van der Heijden PR (1983) Excitation, adaptation and deadaptation of the cAMP-mediated cGMP response in *Dictyostelium discoideum*. *J Cell Biol* 96:347–353
 34. Van Haastert PJM, Van Lookeren Campagne MM, Ross FM (1982) Altered cGMP-phosphodiesterase activity in chemotactic mutants of *Dictyostelium discoideum*. *FEBS Lett* 147:149–152
 35. Van Lookeren Campagne MM, Schaap P, Van Haastert PJM (1986) Specificity of adenosine inhibition of cAMP induced responses in *Dictyostelium* resembles that of the P-site of higher organisms. *Dev Biol* (in press)
 36. Wang M, Schaap P (1985) Correlations between tip dominance, prestalk/prespore pattern and cAMP relay efficiency in slugs of *Dictyostelium discoideum*. *Differentiation* 30:7–14
 37. Weijer CJ, Durston AJ (1985) Influence of cAMP and hydrolysis products on cell type regulation in *Dictyostelium discoideum*. *J Embryol Exp Morphol* 86:19–37
 38. Williams GB, Elder EM, Sussman M (1984) Modulation of the cAMP relay in *Dictyostelium discoideum* by ammonia and other metabolites: Possible morphogenetic consequences. *Dev Biol* 105:377–388
 39. Wurster B, Schubiger K, Wick U, Gerisch G (1977) cGMP in *Dictyostelium discoideum*: oscillations and pulses in response to folic acid and cAMP signals. *FEBS Lett* 76:141–144

Received July 1986 / Accepted in revised form October 2, 1986